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by

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In 1951 a research program was started at the University of Nebraska to compare the developmental effects of thermal neutrons and X rays on different crop seeds. Three crops, barley, tomato and maize, were chosen for additional information involving induced chromosomal aberrations and seedling mutations. The results have been published for barley (Beard *et al.*, 1958, Caldecott *et al.*, 1952, 1954) and for tomato (Yagyu and Morris, 1957). The maize investigations are presented in this bulletin, along with a comparison among the three crops with respect to irradiation effects.

The study on maize was conducted over a four-year period, from 1952 to 1956. All observations except those involving seedling mutations were made on the plants which grew from the irradiated seeds. These will be referred to as the R_1 generation, "R" referring to either X-ray or thermal neutron radiations. For reasons which will be considered later, two generations of self-pollination were necessary for most of the induced seedling mutations to be phenotypically expressed. These were recorded in the R_3 generation.

In the course of the study some technical and biological problems were encountered, which made it difficult to get a complete set of quantitative data over an adequate range of doses in any one series. For this reason several series of irradiations were made, and these will be reported separately.

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LITERATURE REVIEW

This review is confined to studies dealing with the effects of irradiating dormant, dry maize seeds with ionizing radiations. These effects have been measured in terms of germination and survival, enzyme activities, root and plant development, chlorophyll changes, chromosome and pollen abnormalities, transmitted mutations, and yield.

One of the earliest reports on the effects of applying X rays to dormant maize seeds was made by Tascher (1929). He tested various types and varieties of maize and found that, in general, they all had the same tolerance, although a flint type was somewhat more resistant, and a popcorn type less resistant, than the others. The average 50 percent lethal dose for a hybrid was 15,000 r units. Tascher observed chlorophyll-deficient stripes, which had smaller cells than the normal green tissue. Plants from heavily treated seeds were delayed in maturity, and some had approximately 50 percent aborted pollen.

Snyder (1930) observed that when seeds of the Golden Bantam variety of sugary maize were exposed to X rays for up to ten minutes, the seedlings showed accelerated growth over the control. Longer exposure times gave a gradual decrease in the beneficial effects, and a three-hour exposure was lethal to the seedlings. When red aleurone seeds of starchy maize were exposed to X rays for five minutes, the seedlings showed an increased growth rate compared to the control, whereas the same exposure applied to seeds of a yellow dent variety retarded seedling growth.

Malhotra (1932) compared the reactions to X rays of a yellow endosperm and a white endosperm variety of maize by observing germination over an 8-day period. Germination of X-rayed seeds tended to be slower in the white variety than in the yellow variety. There was a general tendency for X rays to decrease the germination, and at three dosages the white variety was more adversely affected than the yellow variety.

Collins and Maxwell (1936), using a wide range of X-ray doses, found no impairment of germination up to 100,000 r units, but complete inhibition at 2,400,000 r units. Delayed killing occurred above 40,000 r units. They reported that cytological observations of root-tip cells made by Longley indicated that the number of dividing cells was less than in the control. Mitoses were abnormal, ranging from lagging chromosomes to undifferentiated masses of chromatin. Maxwell (1938) attempted to determine the mechanism of delayed killing by irradiating 20 different longitudinal zones of the seed while shielding the remaining portion in each case. For single zones, which included the length of the embryo, no delayed killing occurred at or below 100,000 r units, but as the width of the embryo exposed was increased, the delayed killing also increased. The most sensitive volume seemed to be in the center of the embryo.

Bless (1937) found no significant differences in seedling heights when X rays of different wavelengths were used and, in a later report (1938), no significant deviations from control in ear weights on plants grown from seeds which had been exposed to four different doses of X rays of the same wavelength.

Singh (1941) obtained a close relationship between catalase activity and germination of maize seeds exposed to three different doses of X rays, with some stimulation of catalase activity and germination at the lower doses, but a depression of both at the highest dose.

Smith and Kersten (1942) gave maize seeds a dose of soft X rays heavy enough to cause cessation of growth during germination, and found that the seedlings had decreased root length, absence of lateral roots and of adventitious roots around the first internode, and poor development of root tips. There were disturbances also in the cells of different regions of the roots and in the differentiation of regions. However, Kersten *et al.* (1943) observed that, for certain doses of soft X rays, there was a stimulation of primary root growth as measured in 5-day-old seedlings.

Smith (1946) subjected dormant seeds of diploid and tetraploid maize to X-ray doses ranging from 10,000 r to 40,000 r units. The tetraploid seeds showed only slightly higher germination than diploid seeds at most of the doses, but there was considerably less reduction in tetraploid seedling heights. At 30,000 r units, which had most effect both on germination and height, the diploid seedlings averaged 16 percent of their control height, whereas the tetraploid seedlings averaged 28 percent of their control height.

Five reports were published on the effects of exposing dormant maize seeds to the Bikini bomb explosion (Anderson, 1948; Randolph *et al.*, 1948; Anderson *et al.*, 1949; Randolph, 1950; Longley, 1950). Most of the studies involved the F_1 hybrid L289 x I205. Comparisons were made among five lots of seed exposed to the bomb, four lots exposed to X rays and a control lot for each type of radiation. Germination was not affected by any of the treatments, but seedlings from seeds exposed to the higher doses had mottling of the first leaves, with streaks in the later leaves. The Bikini exposures gave more uniform effects than the X-ray treatments with respect to reduction in size and vigor.

The nearly mature plants of the 15,000 r X-ray dose and of the Bikini lot A, which was similar to this X-ray dose in its effects, were classified for sectors of chlorophyll-deficient, morphologically abnormal or dead tissue (Randolph *et al.*, 1948). For the X-ray treatments there were .44 sectors per plant and for the Bikini treatment .60 sectors per plant. Tassel branches of plants from these same treatments were examined for evidences of reciprocal translocations, inversions and deletions. The frequencies of tassel branches with altered chromosomes were, for the X-ray dose, 37.5 percent when the cells observed were in the pachytene stage of meiosis, 24.7 percent for cells in diakinesis and 0.6 percent for cells in anaphase I. The corresponding frequencies for the Bikini lot were 22.4, 13.8 and 0.8 percent, respectively. The majority of aberrations in both treatments were reciprocal translocations.

Anderson *et al.* (1949) found that the frequencies of plants with sectors of abnormal pollen in a portion of the central spike ranged from 29.5 percent for 5,000 r to 70.4 percent for 20,000 r units of X rays, and from 4.0 to 63.1 percent for the different Bikini lots. Only normal pollen was observed in 751 control plants. A more detailed study was made on 124 plants of Bikini lot A, including pollen samples from the central spike and from each lateral branch of the tassels. Of 1782 side branches, 31.5 percent had abnormal pollen, distributed among 183 distinguishable sectors. It was estimated that there were two to three sectors of abnormal pollen per plant, with an average sector size of about two branches.

Anderson (1948) reported that 26 percent of the self-pollinated ears from Bikini lot A and 23 percent from the 15,000 r X-ray dose segregated for endosperm mutations. Preliminary seedling tests on the R_2 progenies disclosed a number of chlorophyll and morphological mutations, but control progenies were not available for comparisons.

Anderson (1948) and Randolph (1950) made crosses between untreated or lightly treated plants and those of the 15,000 r X-ray treatment or Bikini lot A which had chromosomal aberrations or tassel sectors of abnormal pollen. The transmission frequencies of chromosomal aberrations as detected by abnormal pollen in the progenies ranged from 4.7 percent when the Bikini lot A plants were used as males to 7.8 percent for the 15,000 r X-ray plants used as females.

Longley (1950), working with progenies of maize seeds which had been exposed to the atomic bomb at Bikini and to X rays, made a detailed study of 1176 chromosomal breaks at the pachytene stage of meiosis. He found that the combined population of both sources of radiation gave a statistically non-random distribution of breaks among chromosomes and among chromosome arms. Furthermore, in the X-ray group, the six shortest chromosomes had more than the expected number of breaks and the four longest chromosomes fewer than the expected number, whereas in the Bikini group there was no correlation with chromosome length. In the distribution of breaks along chromosome arms by sections beginning at the centromeres, there was, for combined treatments, a statistically significant deviation from randomness, with the breaks tending to concentrate in the 10 microns adjacent to the centromere and falling off in the distal regions.

Singleton (1950) attempted to determine if low doses of radiation had any stimulating effect on maize seeds. An early variety and a short hybrid with the gene **rd** for reduced height were treated with X-ray and gamma-ray doses ranging from 250 r to 2,000 r units. The seeds were planted in latin squares; height measurements were made at weekly intervals and, finally, on the mature plants. None of the doses used gave any significant differences from the control in height or yield.

Eyster (1950) obtained a number of chlorophyll and morphological changes in 73 R_2 progenies after exposing seeds of a sweet corn variety for different lengths of time in a radium-beryllium neutron source. The radiations were a mixture of slow neutrons and gamma rays. Since no controls were available, it is not known how many of these changes were inherent in the variety.

Schmidt and Frolik (1951a, 1951b) irradiated dormant maize seeds of three different F_1 single cross hybrids, including L289 x I205, with 7,500 r units of X rays and with thermal neutrons ranging from 8.4 x 10^{12} to 3.4 x 10^{13} thermal neutrons per square centimeter. Detailed observations were made on the seedlings grown in greenhouse sand benches for a period of 28 days. Maximum seedling stands were not affected by any of the treatments, but there was delayed killing in the L289 x I205 lot for the highest thermal neutron dose, with the stand dropping from a maximum of 98.3 percent to 63.3 percent on the 28th day. The mean height of maize seedlings on each of four measurement dates was lower for irradiated seeds than for the control, with a more severe effect for the higher doses of thermal neutrons. By interpolation it was estimated that the 7500 r X-ray dose had the same effect on seedling height as would 4.2 x 10^{12} thermal neutrons per square centimeter.

Schmidt and Frolik noted a marked increase in exposed leaf number at the two highest thermal neutron doses, and a significant decrease in leaf width with each increase in thermal neutron dosage. The X-ray treatment resulted in a significant decrease in leaf width over that of the control, but the leaves were still significantly wider than in the lowest thermal neutron treatment. For seedlings from seed exposed to thermal neutrons, the first two leaves were heavily mottled, and shorter and narrower than leaves in the control; the third, fourth and fifth leaves were much narrower, and the mottling was confined to streaks in the upper parts of younger leaves; striping on some plants began with the fifth leaf and in some cases persisted to maturity; where narrower leaves occurred, the sixth and later leaves had normal width; and abnormalities often occurred as mirror images of each other in adjacent leaves. A random sampling of tassels indicated that abnormal pollen occurred in entire tassels or in sectors of tassels. The frequency of plants with ears showing sterility and the percentage of sterility for affected ears increased with increase in dosage.

Schwartz (1954) and Schwartz and Bay (1956) have reported some interesting results from subjecting dry maize seeds to high doses of fast neutrons or gamma rays. With both types of radiation, when seedling heights at 10 days were plotted against dose, there was a decrease in height at the lower doses but an increase in height at very high doses (125,000 to 400,000 r units of gamma rays and 15,000 to 25,000 rep of fast neutrons). Cytological examination of the root tips from the high dosage levels showed no cell divisions, and it was hypothesized that growth occurred through cell elongation. Chromosomal breakage was high, but without cell divisions none of the material would be lost. The reversal-in-height phenomenon at high doses persisted after storage of maize seeds for four years (Sicard and Schwartz, 1959).

Haskins and Chapman (1956) observed that treatment of L289 x I205 hybrid seeds with X rays or thermal neutrons gave a reduction in seedling heights and extensive leaf mottling. These seedlings were generally less vigorous than seedlings which were small owing to a shorter growing period or low temperatures. Enzyme assays, made on parts of seedlings above the coleoptilar node, indicated that seedlings reduced in height by irradiation had increases in specific activities of peroxidase, acid phosphatase and polyphenolase, whereas height reductions caused by age or temperature were usually associated with slight decreases in the three types of enzyme activity. However, the authors believed that the treatments did not act directly on the enzymes, since no large or consistent differences in enzyme activities were found for preparations of embryos or etiolated shoots from treated seeds.

Beard *et al.* (1958) made a comparative study of the effects of X rays and thermal neutrons on dormant seeds of four different crops, including the maize hybrid L289 x I205. For maize they used a range of X-ray doses from 4,000 r to 24,000 r and of thermal neutrons from 1.01×10^{13} to 3.26×10^{13} thermal neutrons per square centimeter. They observed that whereas, at 12 days after planting, plants of the highest dosage levels had relatively good survival, at 32 days many plants from seeds exposed to the highest doses had died. On the other hand, at 12 days there was a high correlation between dose of X rays and plant height, but at 32 days the relationship was less distinct. They cautioned that the optimum ages at which height and survival data should be taken may differ, since in their experiments with maize the height measurements would be more accurate at 12 days and the survival data at 32 days. These authors also explained that, at the higher dosages, the surviving plants at the time of pollen shedding may not be representative of the treatments. However, statistically significant correlations with high predictive value were obtained for the relationships between dose and abnormal pollen.

Haskins *et al.* (1958) subjected L289 x I205 dry seeds to doses ranging up to 40,000 r units of X rays and 3.60×10^{13} thermal neutrons per square centimeter. Approximately 65 hours after the seeds had been planted in vermiculite, root tips were fixed and sectioned in paraffin. Cells were measured in two regions, one in the central part of the root 100 microns back from the root-cap line, where there was very little differentiation, and the other in the outer few layers 650 to 850 microns back from the root-cap, where considerable differentiation had occurred. Distinct reductions in root length were observed only at the highest doses. There was no relationship between cell width and treatment, but there were small increases in cell length with increasing dosage for both regions. Mitotic activity was depressed from 14.9 dividing cells per section in the control to 5.8 for 40,000 r units of X rays and to 3.8 for 3.60×10^{13} thermal neutrons per square centimeter. The authors found a tendency for decreased amounts of RNA per root tip with increasing dosage, but no consistent relationship between DNA content and dosage.

Priadcenco *et al.* (1958) found that both X-ray and thermal neutron irradiation of dormant maize seeds tended to cause delays in germination and in flowering. They observed some instances of branched stalks, beginning at the second internode, and female inflorescences at the level of the tiller nodes. The incidence of attack by *Ustilago zeae* was 50 to 60 percent greater for irradiated plants than for control plants, and was of about the same intensity for all doses of irradiation.

Saric (1958b) compared the effects of X-radiation on seeds of inbred lines, single crosses and double crosses. At maturity he found that, while there were differences within each group, the inbred lines were most susceptible to irradiation and the single crosses were usually more susceptible than the double crosses. There was some indication of a stimulating effect of a dose of 2500 r units on height, and number of internodes, leaves and ears. Increasing doses caused a decrease in number of internodes and of leaves, but an increase in number of ears; however, the weight and fertility of the ears were decreased.

Gardner (1961) exposed seeds of the Hays Golden open-pollinated variety of maize to thermal neutron radiation in a study of the effect on yield of combining seed irradiation and mass selection. A dose of 1.28×10^{13} thermal neutrons per square centimeter caused delayed emergence, reduced height, numerous yellow or white leaf stripes, and an incidence of smut which was three times greater than in the control. After four generations of mass selection, accompanied by seed irradiation in the first and third generations, the yield of the irradiated strain was significantly higher than that of the original variety, and only slightly lower than that of the mass-selected control strain.

MATERIALS AND METHODS

The maize material used throughout these studies was a single cross hybrid, L289 x I205, which had been selected primarily for its cytological quality (Randolph, 1948). The seed was obtained from the Nebraska Foundation Seed Division in two different lots, one of which was used in the 1952 irradiation series, and the other in both the 1953 and the 1954 series.

All irradiations were made at the Brookhaven National Laboratory using air-dried, dormant seeds. In 1952 each treatment, including the controls, had the same number of seeds, but in 1953 and 1954 progressively greater numbers of seeds were used with increasing dosage, in order to compensate for the increase in lethality at higher doses in field plantings.

The X radiations were filtered through 1 mm. of aluminum and were applied at 250 KV and 30 ma., with a target distance of 27 cm. The intensity of X rays was approximately 1000 r units per minute for the 1952 and 1953 series, and 1160 r units per minute for the 1954 series. The doses given during the three years ranged from 4,000 r to 32,000 r units.

The thermal neutron irradiations were made in the thermal column of the nuclear reactor, using gold foils exposed with the seeds to calculate the flux values. The total doses during the three-year period ranged from 5.8×10^{12} to 5.1×10^{13} thermal neutrons per square centimeter, with a possible error margin in each case of $\pm$ 15%. Non-irradiated control lots were included with each irradiation series and were handled in the same way as the irradiated lots.

The effects of irradiation on dormant seeds as expressed by seedling development as well as by root-tip chromosomal aberrations were observed only in the 1954 irradiation series.

In order to observe the development of seedlings from irradiated seeds, 100 seeds of each treatment were divided into four replications of 25 seeds each, and were planted on June 4, 1954 in a randomized block design in greenhouse benches containing soil. Plant height was measured from the point of emergence to the tip of the longest extended leaf when the controls for each replication were of approximately the same mean height. Thus, the plants were from 12 to 15 days old in different replications when stands and heights were recorded.

This study was repeated in the fall of 1954, using the same conditions. The seeds were planted on November 13, and two sets of height measurements were taken, the first on November 24 and the second on December 3.

An additional set of data involving seedling development was obtained for the same lots of seeds in June, 1955. Because of the low supply of irradiated seeds by this time, 25 seeds of each treatment were planted in trays of vermiculite, which were set under fluorescent lights in the laboratory. Two successive plantings were made, the first on May 25, and the second on June 6, 1955. Stands and heights were recorded when the plants were eight days old.

The seeds used for cytological observations of the root-tip cells were germinated somewhat over a year after they had been irradiated. They were kept at room temperature during the storage period. On

May 16, 1955, 25 seeds of each treatment were placed between moist blotters in covered plastic dishes, which were then kept in darkness at 20° C. The seeds with the roots attached were fixed in Carnoy's solution when the roots measured between 5 and 9 mm. from the scutellar nodes to the tips. Within this range in length the dividing cells observed were believed to be in the first mitotic cycle after germination of the seeds (based on unpublished studies at this laboratory by Dr. B. H. Beard). Between 59 and 164 hours after the seeds had been placed in blotters, samples were taken every two to three hours.

In preparing root-tip squashes, some roots were pretreated with HCl or an enzyme preparation to dissolve the middle lamellae between cells, but some difficulty was encountered with partial digestion of the chromosomes. Propiono—or acetocarmine was used as a stain, with steaming of slides to increase staining contrast.

All field plantings were made at Lincoln, Nebraska, except for an irradiation series in the fall of 1953, which was grown in Florida. Since difficulties were encountered in getting adequate stands in the field in the 1952 and 1953 series, in 1954 preliminary plantings of 50 seeds from each treatment were made in the greenhouse to check survival against the doses applied.

When the irradiated seeds were planted directly in the field, loss of the more severely affected seedlings often occurred due in part to adverse weather conditions such as strong, drying winds. In order to eliminate this difficulty the X-ray and thermal neutron series irradiated in the spring of 1954 were started in the greenhouse, in plant bands containing soil and set in greenhouse flats. The seedlings were transplanted to the field at the age of two to three weeks, depending on their stage of development.

In 1952 tassel samples for studies of meiotic chromosomal interchanges and pollen abnormalities were collected from a portion of the plants according to their maturity on the collecting dates. The results of these collections indicated that the plants containing the interchanges and possibly other non-identified aberrations tended to develop later than those without the aberrations, and that the sampling dates were not extended over a long enough period in some cases to give a representative picture of the induced interchanges. Hence, in the 1954 series the first 100 plants in each control and the first 200 plants in each treatment were designated for the collection of tassel samples.

The customary cytological procedures for maize were used, with fixation in Farmer's fluid and staining with acetocarmine. During the time that the samples were stored in the fixative at approximately —10° C, a mechanical failure occurred in the refrigeration room and the samples were exposed to temperatures as high as 60° C for a few hours. These samples were difficult to stain with carmine, but Heidenhein's hematoxylin proved satisfactory. They improved after being left at -10° C again for several months, after which propionocarmine stain gave satisfactory results.

In preparing slides for pollen observations an iodine-potassium iodide-gelatin aqueous solution was used as described by Konzak (1952). The pollen grains observed in each of three strips as wide as the microscopic field and as long as the cover slip were recorded for each plant.

In the 1953 irradiation series grown in Florida and the 1954 irradiation series grown at Lincoln self-pollinations were made on as many plants as possible in each treatment for a seedling mutation study. The need to carry the plants through two cycles of self-pollination before being able to detect most of the induced recessive seedling mutations was indicated by Stadler (1930, 1931). He reasoned that the portion of a plant which would be affected by a mutation occurring in a single cell of a mature seed, was not likely to include both the tassel and the ear, so that mutations induced by treatment of mature seeds usually would not segregate in the second generation. Thus, if a seedling mutation is induced by irradiation of the dormant seed, some of the seeds of the R_1 plant may be heterozygous for the mutation if it involved either the tassel or the ear shoot. If the mutation is recessive, segregation will not occur until the R_2 plant is self-pollinated and the R_3 progeny is grown.

In taking seeds from the R_1 plants, it was assumed that a specific induced mutation transmitted through the female gamete would tend to occur in clusters of kernels on the ear, producing a chimeral pattern (Stadler, 1931; Anderson *et al.*, 1949). In an attempt to avoid a duplication of any mutation, the seeds were taken at random from different areas on each R_1 ear. The R_2 seeds from the 1953 Florida irradiation series were planted in the field at Lincoln in 1954, and the R_2 seeds from the 1954 irradiation series were similarly planted at Lincoln in 1955. Observations were made on the R_2 plant progeny rows at intervals during the growing season, and several plants within each progeny were self-pollinated.

From one or more plants which produced selfed seed within each R_2 progeny, an R_3 plant progeny row was grown in sand in greenhouse benches, the 1953 series during the winter of 1954-55, and the 1954 series during the winter of 1955-56. The progenies were allowed to grow for at least two weeks after planting, and during this period notes were taken at least twice on chlorophyll and morphological mutations.

RESULTS

The results of the various irradiation studies are reported separately according to the years in which the seeds were irradiated, namely, the 1952, 1953 and 1954 irradiation series.

Figure 1. Effects of X-irradiation of dormant seeds on stand and height of maize plants 49 days after planting. From left to right (1 row of each treatment): 32,000 r, 24,000 r, 16,000 r, 8,000 r, 4,000 r, control. Irradiated April 1 and planted May 3, 1952.

1952 Irradiation Series

The maize seeds used in the 1952 studies were irradiated with X rays and thermal neutrons on two dates, March 31 and April 1, and each treatment was planted in the field on two dates, May 3 and May 15.

The mature plant stands for each treatment and each planting date are presented in Table 1. Stands of the second planting were, in

Figure 2. Effects of thermal neutron irradiation of dormant seeds on stand and height of maize plants 49 days after planting. From left to right (1 row of each treatment): 3.1×10^{13}, 1.9×10^{13}, 1.0×10^{13} and 5.8×10^{12} thermal neutrons per square centimeter. Irradiated April 1 and planted May 3, 1952.

Table 1.—Mature stands of maize plants from seeds irradiated and planted on two dates in 1952, 250 seeds per treatment.

Treatment	Date of irradiation: March 31 — Date of planting: May 3 — No. of plants	March 31 — May 3 — % of control	March 31 — May 15 — No. of plants	March 31 — May 15 — % of control	April 1 — Date of planting: May 3 — No. of plants	April 1 — May 3 — % of control	April 1 — May 15 — No. of plants	April 1 — May 15 — % of control
Control	169	100.0	199	100.0	235	100.0	200	100.0
X rays								
4,000 r	222	131.4	156	78.4	221	94.0	198	99.0
8,000 r	197	116.6	48	24.1	192	81.7	53	26.5
16,000 r	37	21.9	1	0.5	48	20.4	6	3.0
24,000 r	12	7.1	2	1.0	9	3.8	3	1.5
32,000 r	6	3.6	4	2.0	7	3.0	2	1.0
Thermal neutrons per square centimeter								
5.8×10^{12}					206	87.7	198	99.0
1.0×10^{13}					207	88.1	233	116.5
1.1×10^{13}	207	122.5	179	89.9				
1.7×10^{13}	190	112.4	193	97.0				
1.9×10^{13}					219	93.2	213	106.5
2.5×10^{13}	207	122.5	99	49.7				
3.1×10^{13}					173	73.6	8	4.0
4.2×10^{13}	93	55.0	0	0.0				

general, lower than those of the first planting because of adverse weather conditions at the time of emergence. This was particularly true at the higher doses of both X rays and thermal neutrons. However, for the first planting date there was a marked drop in stands between 8,000 r and 16,000 r units for X rays at both irradiation dates and between 2.5 x 10^{13} and 4.2 x 10^{13} thermal neutrons per square centimeter at the first irradiation date. This information was used in setting the dose ranges for future experiments.

The stand of the control lot accompanying the March 31 treatment planted May 3 (67.6 percent) was considerably lower than that of the May 15 planting (79.6 percent) for some unknown reason. The reduction in stand for the control lots of the April 1 treatments from 94 percent on the May 3 planting date to 80 percent on the May 15 planting date was probably a reflection of differing environmental conditions. The effects of various treatments are pictured in Figures 1 and 2.

Observations were made on the plants from the seedling stage until the time of tasseling for color changes, slashed leaves, branched main stalks and any other irregularities. The color changes included white, cream, yellow, yellow-green, light-green, medium-green and blue-green sectors. Some of the sectors had a mottled appearance, with green spots or blotches on a lighter background. Occasionally a leaf had sectors of different colors in identical areas on the upper and lower surfaces. The sectors varied in size and in the number of leaves which they included. An example of an unusually extensive yellow sectoring which included most of the leaves, the tassel and the ear shoot, is shown in Figure 3.

Slashed leaves were thought to result from the action of the wind on streaks of irradiation-induced dead tissue, although there was a small amount of slashing in the control series. In some cases the slashes occurred on both sides of the midrib, whereas in other cases they were confined to one side. The plant in Figure 3 has slashes in its lower leaves.

Table 2.—Frequencies of plants with color chimeras and slashed leaves from seeds irradiated April 1 and planted May 3, 1952 (notes taken 45 days after planting).

Treatment	No. of plants	Color chimeras		Slashed leaves	
		No.	%	No.	%
Control	235	17	7.2	4	1.7
X rays					
4,000 r	221	18	8.1	6	2.7
8,000 r	192	35	18.2	25	13.0
16,000 r	48	7	14.6	9	18.8
Thermal neutrons per square centimeter					
5.8 x 10^{12}	206	24	11.7	15	7.3
1.0 x 10^{13}	207	38	18.4	22	10.6
1.9 x 10^{13}	219	45	20.5	60	27.4
3.1 x 10^{13}	173	32	18.5	30	17.3

Figure 3. A maize plant from a thermal neutron-irradiated seed, two months after planting, showing extensive yellow sectoring including tassel. Leaf slashes are also evident.

There was a general tendency for irradiation to increase the frequencies of color chimeras and of slashed leaves. Observations on 45-day-old plants from seeds irradiated April 1 and planted May 3 are presented in Table 2. The 24,000 r and 32,000 r doses of X rays are not included, since the stands were not sufficient to give a reliable frequency of the abnormalities.

Branching of the main stalk to give two and, in one case, three tassels, was observed in a total of 57 plants, of which 19 occurred in one thermal neutron treatment (1.9×10^{13} thermal neutrons per square centimeter). No plants with branched stalks were observed in any of the control series. An example of "twin stalks" is shown in Figure 4.

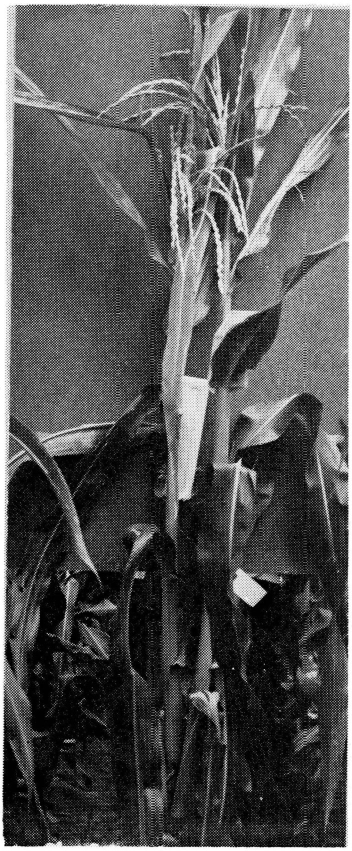


Figure 4. A maize plant from an irradiated seed showing branching of the main stalk into two parts, with tassel and ear shoot development on each of the two branches.

Table 3.—Interchange frequencies at microsporogenesis for different doses of X rays and thermal neutrons and for two planting dates, 1952 series. Data for the two irradiation dates have been combined in the X-ray series and in the control lots.

Treatment	Date of planting: May 3			Date of planting: May 15		
	No. of plants sampled	Interchange frequency[1]		No. of plants sampled	Interchange frequency	
		No.	Per 100 plants		No.	Per 100 plants
Control	61	0	0.0	74	1	1.3
X rays						
4,000 r	118	11	9.3	84	16	19.0
8,000 r	133	33.5	25.2	86	58.5	68.0
16,000 r	48	16.5	34.4		(Inadequate stand)	
Thermal neutrons per square centimeter						
5.8×10^{12}	75	14	18.7	46	4.5	9.8
1.0×10^{13}	84	14.5	17.3	80	14	17.5
1.1×10^{13}	85	11.5	13.5	52	13.5	26.0
1.7×10^{13}	85	18.5	21.8	67	21	31.3
1.9×10^{13}	107	45.5	42.5	124	94	75.8
2.5×10^{13}	87	49	56.3	47	47	100.0
3.1×10^{13}	120	82	68.3		(Inadequate stand)	
4.2×10^{13}	68	48.5	71.3		(No stand)	

[1]Using a ring-of-4 as a basis. A ring-of-6 is counted as 1.5 rings-of-4, a ring-of-8 as two rings-of-4, etc.

The frequencies of interchanges (reciprocal translocations) observed at microsporogenesis are presented in Table 3. The X-ray doses of 24,000 r and 32,000 r units are excluded because of insufficient stands. Since there were no statistically significant differences between the two irradiation dates for any of the X-ray treatments shown or the control lots, the data for the two dates have been combined in each case.

There was a statistically significant increase in interchange frequency for the May 15 planting date compared to the May 3 planting date at the following doses: 4,000 r and 8,000 r units of X rays, and 1.9×10^{13} and 2.5×10^{13} thermal neutrons per square centimeter. Since some thermal neutron treatments and the control treatment showed no statistically significant increases at the second planting date, the possibility of environmental causes is eliminated. The storage of the seeds after irradiation before planting should be considered in view of recent findings (Adams and Nilan, 1958). However, since the seeds were stored in air and since the interval between irradiation and the first planting date was 33 or 34 days, it does not seem likely that an extra 12 days of storage before the second planting would have produced the striking increases in interchange frequency shown by 8,000 r units and by 1.9×10^{13} and 2.5×10^{13} thermal neutrons per square centimeter.

The following explanation would seem to be more feasible. As was mentioned in Materials and Methods (p. 9), the plants were sampled according to stage of maturity on the collecting dates. Because of reduced stands, the collecting period for the May 15 planting extended over a longer period of time than that for the May 3 planting. For doses of 1.7×10^{13}, 1.9×10^{13} and 2.5×10^{13} thermal neutrons per square centimeter, the collecting period for the May 3 planting was 3 days and for the May 15 planting, 7 to 11 days. The collecting period for the 8,000 r X-ray treatment extended over 4 days for the May 3 planting and over 27 days for the May 15 planting. Furthermore, for the X-ray dose, only 34 percent of the plants from the May 3 planting were sampled, whereas 85 percent of the plants from the May 15 planting were sampled. This was owing to the difference in stands, which were 389 plants for the first planting date and 101 plants for the second planting date. The types of interchanges scored for the different collecting dates indicated that plants with interchanges tended to mature later than those without interchanges. This delay in maturity might have been due also to other aberrations stemming from the multiple breaks. Hence, the short collecting periods for the first planting date would not have included the more aberrant plants. This is indicated in Table 4, where the different types of interchanges are presented for the irradiation doses which had the most striking differences between planting dates. In addition to a greater number of plants with rings-of-four

Table 4.—Frequencies per 100 plants of different types of interchanges for doses of X rays and thermal neutrons with significant differences between planting dates.

Treatment and planting date	Type of interchange										
	No. of plants	Ring-of-4	Ring-of-6	Ring-of-8	Two rings-of-4	Two pseudo-isochromosomes[1]	Two pseudo-isochromosomes + ring-of-4	Ring-of-4 + ring-of-6	Ring-of-4 + ring-of-8	Ring-of-4 + ring-of-10	Others[2]
X rays, 8,000 r units											
May 3	133	21.1	0.8	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0
May 15	86	30.2	3.5	3.5	3.5	2.3	1.2	1.2	1.2	1.2	1.2
Thermal neutrons, 1.9 x 10^{13} and 2.5 x 10^{13} per square centimeter											
May 3	194	26.3	3.1	0.5	7.2	1.0	0.0	0.5	0.0	0.0	0.0
May 15	171	33.3	6.4	1.2	13.5	1.2	0.6	1.2	0.6	0.6	0.6

[1] Interchange involving opposite arms of homologous chromosomes.
[2] These include:
- A. Ring-of-4 + one pseudo-isochromosome + a trivalent involving the other pseudo-isochromosome
- B. Ring-of-6 + two rings-of-4
- C. Three rings-of-4
- D. Ring-of-4 involving one pseudo-isochromosome + one pseudo-isochromosome
- E. Ring-of-8 + two pseudo-isochromosomes
- F. Ring-of-4 + ring-of-6 + two pseudo-isochromosomes

at the second planting date, there were increases in the more complex interchanges or combinations of different interchanges.

In spite of the bias attributed to sampling technique, it is apparent from Table 3 that the interchange frequency has been increased by irradiation of dormant maize seeds, and that at the higher doses of thermal neutrons used, a high proportion of plants would be expected to carry an interchange of some type.

Meiotic cells showing some of the interchange configurations observed are reproduced in Figures 5-12. The combination of a ring-of-four and a chain-of-ten (Figure 10) involved a minimum of seven breaks. It is of interest to note that this combination was recovered once in both the X-ray and the thermal neutron series (see Table 5).

The breaks and exchanges involving opposite arms of homologous chromosomes, which result in "pseudo-isochromosomes," have been discussed elsewhere (Morris, 1955).

The frequencies of the different types of interchanges produced by X-ray and thermal neutron irradiations are presented in Table 5. There is a statistically significant difference between the combined doses of X rays and of thermal neutrons with respect to the frequency of two rings-of-four, but the frequencies of the other types of interchanges do not differ significantly. However, there is a trend toward a higher frequency of interchanges in the thermal neutron series and, if the different types of interchanges are combined, the frequency of 33.1 percent for the combined thermal neutron doses is significantly higher than that for the combined X-ray doses, which is 25.9 percent.

Observations on the frequencies of abnormal pollen at different doses of X rays and thermal neutrons are presented in Table 6. The data include the two irradiation dates but only the May 3 planting date. Pollen grains were classified as abnormal if they were small, empty or only partially filled with starch.

Since the pollen samples were collected according to the maturity of the plants on the collection dates, the pollen data are subject to the same bias as the data on interchange frequencies, but to a lesser extent. Since a larger number of plants were sampled for pollen observations, the first 100 plants sampled in order within the row, or all available below 100, were used for pollen observations.

The control groups for the two irradiation dates did not differ significantly; hence, the average frequency of abnormal pollen in the 135 control plants, based on 60,740 pollen grains, was 6.3 percent. Allowing for this frequency due to causes other than irradiation, the amount of induced pollen abnormalities ranged from 8 percent for the lowest dose of X rays to 75 percent for the highest dose of thermal neutrons. However, there was not a consistent increase with dosage in the case of the thermal neutrons, which may be related to the sampling techniques. At the higher doses, particularly, the abnormal pollen may

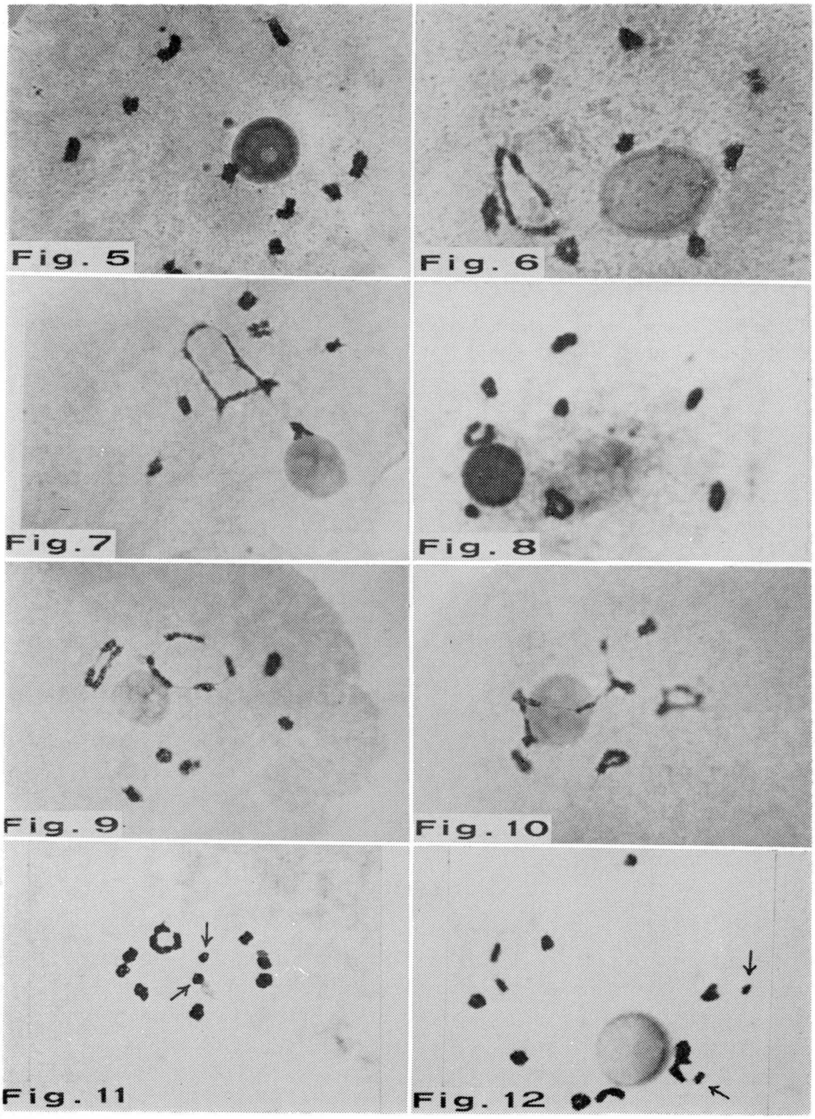
Table 5.—Frequencies per 100 plants of different types of interchanges induced by various doses of X rays and thermal neutrons, 1952 irradiation series. Two irradiation dates and two planting dates combined.

Treatment	Type of interchange										
	No. of plants	Ring-of-4	Ring-of-6	Ring-of-8	Two rings-of-4	Two pseudo-isochromosomes[1]	Two pseudo-isochromosomes + ring-of-4	Ring-of-4 + ring-of-6	Ring-of-4 + ring-of-8	Ring-of-4 + ring-of-10	Others[2]
Control	135	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
X rays											
4,000 r	202	11.9	0.0	0.0	0.5	0.5	0.0	0.0	0.0	0.0	0.0
8,000 r	219	24.7	1.8	1.4	2.3	0.9	0.5	0.5	0.5	0.5	0.5 (A)
16,000 r	54	16.7	3.7	0.0	7.4	0.0	0.0	0.0	0.0	0.0	0.0
24,000 r	22	27.3	4.5	0.0	13.6	0.0	0.0	0.0	0.0	0.0	4.5 (B)
32,000 r	12	41.7	0.0	0.0	0.0	8.3	0.0	0.0	0.0	0.0	0.0
Combined X-ray doses	509	19.3	1.4	0.6	2.6	0.8	0.2	0.2	0.2	0.2	0.4
Thermal neutrons per square centimeter											
5.8×10^{12}	121	9.1	0.8	0.0	2.5	0.0	0.0	0.0	0.0	0.0	0.0
1.0×10^{13}	164	14.0	0.6	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0
1.1×10^{13}	137	12.4	0.7	0.0	1.5	0.0	0.0	0.7	0.0	0.0	0.0
1.7×10^{13}	152	17.1	3.3	0.0	0.7	0.7	0.0	0.0	0.0	0.0	0.7 (C)
1.9×10^{13}	231	29.9	4.8	0.4	9.1	1.3	0.4	0.0	0.4	0.0	0.4 (D)
2.5×10^{13}	134	29.1	4.5	1.5	11.9	0.7	0.0	2.2	0.0	0.7	0.0
3.1×10^{13}	120	36.7	1.7	1.7	9.2	0.0	2.5	0.0	0.0	0.0	0.8 (E)
4.2×10^{13}	68	33.8	5.9	1.5	10.3	0.0	0.0	0.0	0.0	0.0	1.5 (F)
Combined thermal neutron doses	1127	22.4	2.8	0.5	5.6	0.4	0.4	0.4	0.1	0.1	0.4

[1] Interchange involving opposite arms of homologous chromosomes.

[2] These include:

A. Ring-of-4 + one pseudo-isochromosome + a trivalent involving the other pseudo-isochromosome
B. Ring-of-6 + two rings-of-4
C. Three rings-of-4
D. Ring-of-4 involving one pseudo-isochromosome + one pseudo-isochromosome
E. Ring-of-8 + two pseudo-isochromosomes
F. Ring-of-4 + ring-of-6 + two pseudo-isochromosomes



Figures 5-12. Photomicrographs of microsporocytes of maize showing interchange configurations at diakinesis. Figure 5—A normal cell with ten bivalents. Figure 6—A ring-of-six and seven bivalents. Figure 7—A ring-of-eight and six bivalents. Figure 8—Two rings-of-four and six bivalents. Figure 9—A ring-of-four, ring-of-six and five bivalents. Figure 10—A chain-of-ten, ring-of-four and three bivalents. Figure 11—A ring-of-four, pseudo-isochromosomes for chromosome 6 (marked with arrows) and seven bivalents. Figure 12—A ring-of-four with one pseudo-isochromosome involved (arrow), the other pseudo-isochromosome free (arrow), and eight bivalents.

be due to types of chromosome aberrations other than interchanges, which were the only type recorded.

The high standard error values for the 16,000 r X-ray dose indicate a greater range of variability than those for the highest thermal neutron doses. For the 16,000 r X-ray dose 20 out of 55 plants for the combined irradiation dates had approximately 95 percent normal pollen, whereas the other 35 plants had frequencies of abnormal pollen ranging from 30 to 95 percent. On the other hand, the highest thermal neutron dose had no plants with largely normal pollen, and only 11 of the 59 plants had less than 75 percent abnormal pollen.

Table 6.—Frequencies of abnormal pollen for different doses of X rays and thermal neutrons, two irradiation dates, May 3 planting date, 1952 irradiation series.

Treatment	Date of irradiation			
	March 31		April 1	
	No. of plants	% abnormal pollen Mean $\pm s_{\bar{x}}$	No. of plants	% abnormal pollen Mean $\pm s_{\bar{x}}$
Control	59	$6.1 \pm .57$	76	$6.4 \pm .38$
X rays				
4,000 r	101	15.5 ± 1.44	90	14.1 ± 1.27
8,000 r	100	33.7 ± 2.81	100	34.3 ± 2.62
16,000 r	23	36.3 ± 6.59	32	44.8 ± 5.44
Thermal neutrons per square centimeter				
5.8×10^{12}			90	21.5 ± 1.98
1.0×10^{13}			96	53.5 ± 2.51
1.1×10^{13}	99	26.5 ± 2.43		
1.7×10^{13}	100	50.3 ± 2.27		
1.9×10^{13}			97	75.3 ± 1.98
2.5×10^{13}	106	68.7 ± 2.14		
3.1×10^{13}			96	73.6 ± 2.17
4.2×10^{13}	59	80.7 ± 1.79		

1953 Irradiation Series

Spring Series

After the experience with the 1952 irradiation series an experiment was planned for the spring of 1953 with two modifications. The doses were narrowed to a range of from 4,000 r to 12,000 r units of X rays and from 7.8×10^{12} to 3.5×10^{13} thermal neutrons per square centimeter. Furthermore, the number of seeds irradiated was increased with each dosage increment, in order to compensate for induced mortality and to get approximately the same mature stands at all doses.

The five X-ray treatments and eight thermal neutron treatments, along with an untreated lot, were each divided into two groups of equal size, and planted directly in the field, one group on May 16 and the other group on May 29.

Emergence was good at all doses for both planting dates but, owing to adverse weather conditions, especially hot, drying winds, which occurred shortly after emergence, there was almost complete killing for all treatments except the lowest doses of X rays and thermal neutrons. Hence, no data were obtained for the 1953 spring series.

Fall Series

Because of the failure to obtain any mature plants from the 1953 spring series, seeds were irradiated in the fall of 1953 and planted in the field in Florida. The X radiations were applied on October 13 and the thermal neutron radiations on November 3 and 4. All the seeds were planted in Florida in the latter part of November.

When stands were taken on February 1, 1954, before the information on the fluxes for the thermal neutron doses had been received, it was found that, with the exception of the lowest dose, all the thermal neutron treatments gave drastic reductions in stand, which had not been anticipated with the doses requested. Upon investigation it was found that the flux values for the foils exposed with the seed were higher than intended for some inexplicable reason. As a result the total doses were much higher than desired.

The mature stands for the Florida irradiation series are presented in Table 7. The X-ray series gave satisfactory stands for sampling even at the highest dose, where approximately 50 percent killing occurred, but the stands in the thermal neutron series fell off markedly after the lowest dose. The dose of 2.7 x 10^{13} thermal neutrons per square centimeter appears out of line when compared with the other doses with respect to stand, and this was found to be due to an aberrance in the nuclear reactor, which was detected by dosage-measuring instruments.

In order to obtain data on induced seedling mutations, as many R_1 plants as possible in each treatment were self-pollinated. At harvest time 20 seeds (or less where seed set was poor) were taken at random from different areas of each ear, in order to avoid the possibility of obtaining a specific mutation in more than one seed as explained in Materials and Methods (p. 10).

Table 7.—Mature plant stands for different doses of X rays and thermal neutrons, 1953 fall series (Florida).

Treatment	No. of seeds	Mature plant stand	
		No.	%
Control	600	523	87.2
X rays			
6,000 r	708	627	88.6
8,000 r	880	714	81.1**
10,000 r	1200	835	69.6**
12,000 r	1600	755	47.2**
Thermal neutrons per square centimeter			
2.2 x 10^{13}	640	553	86.4
2.7 x 10^{13}	684	64	9.4**
3.5 x 10^{13}	880	196	22.3**
5.1 x 10^{13}	1200	7	0.6**

**Significant decrease from control stand at the 1% level of probability.

Table 8.—Frequencies of seedling and mature plant mutations in the R_2 generation from irradiation of dormant seeds, 1953 fall series (Florida).

Treatment	No. of progenies	Seedling mutations		Mature plant mutations	
		No.	Per 100 progenies	No.	Per 100 progenies
Control	352	81	23.0	13	3.7
X rays					
6,000 r	402	102	25.4	16	4.0
8,000 r	367	106	28.9	9	2.5
10,000 r	271	84	31.0*	5	1.8
12,000 r	192	43	22.4	3	1.6
Thermal neutrons per square centimeter					
2.2×10^{13}	294	69	23.5	8	2.7

*Significant increase over control frequency at the 5% level of probability.

In 1954 13 seeds (in a few cases less) from each R_1 ear were planted in ear progeny rows in the field at Lincoln, Nebraska. Observations were made on the R_2 plants throughout their development. The frequencies of mutations for the R_2 generation are presented in Table 8. In the thermal neutron series only the lowest dose of thermal neutrons had sufficient progenies to make a quantitative determination of mutation frequency.

It is evident from the frequency of mutations in the control lot that the single cross hybrid used in these studies was heterozygous for a number of recessive genes the effects of which were expressed in the R_2, the segregating generation for the hybrid. The X-ray dose of 10,000 r units was the only treatment which had a statistically significant increase over the control in frequency of progenies with seedling mutations, and there were no significant differences between the control and any of the treatments with respect to mature plant mutations. These observations would indicate that most of the mutations observed in the R_2 generation were not irradiation-induced. However, there were significant differences between the control and the 10,000 r X-ray dose in frequencies of virescent and necrotic seedlings, and between control and the thermal neutron dose in the frequency of virescent seedlings (Table 10).

The mature plant mutations in the R_2 generation, which are included in Table 8, consisted of striped, dwarf-like, male-sterile and chlorophyll-mutable plants. Two progenies derived from irradiated seeds segregated for brown midrib and one progeny each segregated for tasselseed and tasselless. However, an overall comparison with the control indicated that, in general, the mature plant mutations were not irradiation-induced.

The 12,000 r X-ray dose had more defective seeds than any of the other treatments, and these seeds for the most part did not germinate. In this event any mutation carried by a defective seed would be lost, and this fact could contribute to the lower mutation frequency for this dose than for the lower doses.

Where possible, one or more plants in each R_2 progeny were self-pollinated on the assumption, as explained in Materials and Methods (p. 10), that most of the irradiation-induced mutations would not be expressed until the R_3 generation. The R_3 progenies for the control, for the 6,000 r and 10,000 r doses of X rays, and for the lowest thermal neutron dose were grown in sand or soil benches in the greenhouse during the winter of 1954-55 for a classification of seedling mutations. The results of these observations are given in Table 9. Only one ear from each R_2 progeny was included for both the treatments and the control, to avoid classifying an induced mutation more than once, and also to avoid classifying mutations inherent in the single cross hybrid more than once per R_1 plant. Progenies having less than ten plants were excluded from the summary of data.

There is no statistically significant increase in mutations for the 6,000 r units of X rays compared to the control, but the 10,000 r units of X rays and the thermal neutron dose both show similar and marked increases over the control.

The types of seedling mutations observed in the R_2 and R_3 generations are summarized in Table 10. Many of these were lethal and consisted of chlorophyll changes or necroses. The mutable phenotype consisted of leaves with a light background, such as cream, yellow or yellow-green, overlaid with green spots or streaks.

Comparisons in the R_2 between the control and radiation treatments with respect to specific mutations have already been made (p. 24). In the R_3 generation statistically significant increases over the control were obtained for cream seedlings with the three irradiation treatments, for glossy with the two X-ray treatments, for yellow-green and pale-green with the thermal neutron treatment and the 10,000 r X-ray dose, for virescent, fused leaves and twisted leaves with the 10,000 r X-ray dose, and for striate, striped and necrotic or chlorotic with the thermal neutron dose.

In comparisons of the R_2 and R_3 generations, the overall increase

Table 9.—Frequencies of seedling mutations in the R_3 generation from irradiation of dormant seeds, 1953 fall series (Florida).

Treatment	No. of progenies	Seedling mutation frequency	
		No.	Per 100 progenies
Control	292	85	29.1
X rays			
6,000 r	328	111	33.8
10,000 r	146	89	61.0**
Thermal neutrons per square centimeter			
2.2×10^{13}	238	143	60.1**

**Significant increase over control frequency at the 1% level of probability.

Table 10.—Percentages of R_2 and R_3 progenies with different types of seedling mutations, 1953 irradiation series.

Treatment	Type of mutation													
	White	Cream	Yellow	Yellow-green	Pale-green	Virescent	Striate	Stripes	Mutable	Necrotic or chlorotic	Glossy	Dwarf	Fused leaves	Others
Control														
for R_2	5.4	2.3	1.4	6.0	0.6	2.0	0.3	2.3	0.3	2.3	0.3	0.0	0.0	0.0
for R_3	3.8	3.4	1.4	11.6	0.0	6.5	0.0	0.3	0.6	0.7	0.0	0.3	0.3	0.0
X rays														
6,000 r R_2	4.0	2.7	0.5	8.0	0.5	1.0	0.7	1.2	0.5	3.7	1.7	0.0	0.0	0.5[1]
R_3	1.8	9.5	0.3	9.5	0.3	5.8	0.6	1.2	0.6	0.6	2.7	0.3	0.3	0.3[2]
10,000 r R_2	4.4	0.4	3.0	5.2	0.0	8.1	0.0	1.5	0.0	7.4	0.7	0.0	0.0	0.0
R_3	4.1	6.8	1.4	21.9	2.1	15.1	0.0	0.0	1.4	1.4	1.4	0.7	3.4	1.4[2]
Thermal neutrons per square centimeter														
2.2×10^{13} R_2	5.4	2.0	2.0	5.8	0.0	6.1	0.7	1.0	0.0	0.0	0.0	0.0	0.0	0.3[3]
R_3	4.6	7.6	2.1	16.4	1.7	4.6	8.4	6.7	1.7	3.4	0.8	1.3	0.4	0.4[4]

[1] Albescent-like
[2] First leaf twisted
[3] Banded
[4] Abnormal emergence

in seedling mutation frequency for the R_3 control compared to the R_2 control was not statistically significant, but there was a significant increase for the yellow-green and virescent mutations. Since the R_2 plants were grown in the field and the R_3 plants in the greenhouse, it is possible that more of these environment-sensitive mutations were detected under greenhouse conditions than under field conditions.

As for the effects of different radiation doses in the R_3 versus the R_2, significant overall increases were obtained in the R_3 for the three treatments which were tested in both generations. Considering specific mutations, there were significant increases in the R_3 for cream seedlings with the three irradiation treatments, for virescent with the two X-ray doses, for yellow-green, pale-green and mutable with the 10,000 r X-ray dose and the thermal neutron dose, for fused leaves and twisted leaves with the 10,000 r X-ray dose and for striate, striped and necrotic or chlorotic with the thermal neutron dose.

1954 Irradiation Series

A final attempt was made in the spring of 1954 to obtain a range of X-ray and thermal neutron doses which would permit quantitative determinations of irradiation effects.

Information concerning the doses applied to dormant seeds of the L289 x I205 single cross hybrid and dates of irradiation is given in Table 11. A range of thermal neutron doses from 1.6×10^{13} to approximately 3×10^{13} thermal neutrons per square centimeter was sought, but when the flux values were obtained after the R_1 generation had been grown, it was found that the range was narrower, with only a small difference between the two highest doses.

Table 11.—Date of irradiation of dormant seeds and mature stands of plants from irradiated seeds, 1954 irradiation series.

Treatment	Date of irradiation	No. of seeds planted for the field	Mature stand (October 14, 1954)	
			Number	%
X rays				
Control		350	321	91.7
5,800 r	April 30, 1954	700	645	92.1
8,120 r	April 30, 1954	800	710	88.7
10,440 r	April 30, 1954	1250 (1120)[1]	924	82.5**
11,948 r	April 30, 1954	1500 (1241)	1031	83.1**
Thermal neutrons per square centimeter				
Control		350	308	88.0
1.6×10^{13}	April 1, 1954	800 (786)	598	76.1**
2.3×10^{13}	March 31, 1954	1250 (1128)	728	64.5**
2.5×10^{13}	March 21, 1954	1500 (1370)	745	54.3**

[1] Number in parentheses excludes the plants thinned where there were two plants per band and should be used for comparison with the mature stand.

**Significant decrease from control stand at the 1% level of probability.

Adequate seeds were provided for each dosage to make observations on root-tip chromosomal aberrations and seedling stands and heights, in addition to aiming for field stands of at least 500 mature plants for each irradiation treatment. Thus, whereas 1100 seeds were provided for each control, 1900 seeds were designated for the highest X-ray dose and 2500 seeds for the dose of 2.5 x 10^{13} thermal neutrons per square centimeter.

Before making the planting for the field, a preliminary planting of each treatment was made in the greenhouse to check on survival at the doses applied. The portion of each treatment designated for the field was initially planted in bands of soil in greenhouse flats on May 20 and 21, 1954. The flats were kept in the greenhouse until the plants were transferred to the field on June 9 through 11, except for the highest thermal neutron dose, which was transplanted in part on June 18 owing to the slow development of some plants.

At the higher doses, two seeds per band were planted in some cases. Where both plants emerged they were randomly thinned to one plant. Allowance was made for the discarded plants in presenting frequencies of mature plant stands, as shown in Table 11.

In comparisons with controls, there were statistically significant decreases in stand for the two highest X-ray doses and for all the thermal neutron doses (using a chi-square test for independence in an R x 2 contingency table). However, the highest thermal neutron dose had a stand above 50 percent, and each irradiated lot had a mature stand of over 500 plants.

Seedling Development

Three different sets of data involving seedling development were obtained in the course of a year, as described on page 8. The first test was made in June, 1954, and the results are presented in Table 12. A second test was made in November, 1954, and measurements of

Table 12.—Irradiation effects on dormant maize seeds expressed as plant height at two weeks after planting on June 4, 1954, 100 seeds per treatment.

Treatment	No. of plants	Plant height	
		Mean (cm.) $\pm s_{\bar{x}}$	% control
X rays			
Control	97	35.9 ± .71	
5,800 r	90	29.1 ± .80	81.1
8,120 r	97	28.5 ± .79	79.4
10,440 r	86	25.6 ± .64	71.3
11,948 r	87	21.2 ± .78	59.1
Thermal neutrons per square centimeter			
Control	93	35.9 ± .68	
1.6 x 10^{13}	87	14.8 ± .41	41.2
2.3 x 10^{13}	84	14.0 ± .55	39.0
2.5 x 10^{13}	87	9.3 ± .40	25.9

Table 13.—Irradiation effects on dormant maize seeds expressed as plant height for 11- and 20-day-old seedlings from seeds planted November 13, 1954, 100 seeds per treatment.

Treatment	11 days after planting			20 days after planting		
	No. of plants	Mean height (cm.) ± s$\bar{x}$	% control	No. of plants	Mean height (cm.) ± s$\bar{x}$	% control
X rays						
Control	95	12.8 ± .28		95	36.8 ± .61	
5,800 r	92	8.7 ± .22	68.0	93	25.9 ± .59	70.4
8,120 r	96	7.5 ± .22	58.6	94	22.7 ± .58	61.7
10,440 r	96	5.2 ± .22	40.6	96	13.9 ± .72	37.8
11,948 r	97	4.2 ± .23	32.8	98	9.8 ± .48	26.6
Thermal neutrons per square centimeter						
Control	97	12.2 ± .34		97	36.7 ± .68	
1.6 x 10^{13}	94	4.4 ± .14	36.1	94	10.3 ± .50	28.1
2.3 x 10^{13}	92	4.1 ± .14	33.6	92	9.2 ± .48	25.1
2.5 x 10^{13}	96	3.5 ± .08	28.7	96	6.2 ± .38	16.9

plant height for 11- and 20-day-old seedlings are given in Table 13. The third test, involving a smaller number of seeds, was made in May and June, 1955, over a year after the seeds had been irradiated, and the results are presented in Table 14.

Analyses of variance on the mean plant heights of each treatment were made for the three tests and, in the case of the November, 1954, test, for the two dates of measurement. In each case an F test indicated a significant difference among treatment means. When Duncan's multiple range test (1955) was applied to the data, there was a significant difference at the 1 percent level of probability between the control and each radiation treatment in all three tests. In the X-ray series there were significant differences between each of the two lowest doses and the highest dose, but some of the other treatment combinations were not significantly different. In the thermal neutron series there

Table 14.—Irradiation effects on dormant maize seeds expressed as plant height for 8-day-old seedlings from seeds planted May 25 and June 6, 1955, 50 seeds per treatment.

Treatment	No. of plants	Height of 8-day-old seedlings (two planting dates combined)	
		Mean (cm.) ± s$\bar{x}$	% control
X rays			
Control	48	12.4 ± .60	
5,800 r	49	8.6 ± .46	69.4
8,120 r	47	8.3 ± .33	66.9
10,440 r	46	6.7 ± .35	54.0
11,948 r	47	4.7 ± .25	37.9
Thermal neutrons per square centimeter			
Control	47	13.8 ± .46	
1.6 x 10^{13}	48	6.0 ± .23	43.5
2.3 x 10^{13}	48	4.8 ± .24	34.8
2.5 x 10^{13}	48	4.9 ± .21	35.5

were no significant differences between any pairs of treatments for the first measurement date of the second test (Table 13) or for the third test (Table 14), but most of the other combinations of treatments showed significant differences.

Since the first two tests were made under comparable conditions except, possibly, for the light intensity at the different seasons, they can be compared by expressing the mean height for each treatment in terms of its control. If these values in the first test are compared with the 11-day-old seedling measurements of the second test, it is apparent that, in the X-ray series, plant height is depressed to a greater extent in the second test than in the first test. On the other hand, in the thermal neutron series, there is considerably less difference between the two tests. This may be an indication of the differences in the effects of storing X-ray and thermal neutron-irradiated seeds. The differences are not as pronounced in the third test, which represents a considerably longer storage period, but it is not justifiable to compare this test with the other two in view of the different environmental conditions used in growing the seedlings.

When the 20-day-old seedling measurements were made in the second test (Table 13), some delayed killing was apparent at the higher doses. Thus, at the highest X-ray dose, 20 of the plants measured appeared dead, and at the highest thermal neutron dose 28 plants were believed dead. In none of the three tests were the plants grown for a long enough period to detect all the delayed killing as a result of irradiation, so no conclusions should be made concerning the plant stands in the different tests.

Chromosomal Aberrations

Root-tip meristematic cells believed to be in the first mitotic cycle after germination of the dormant, irradiated seeds, were observed at

Table 15.—Frequencies of anaphase bridges, fragments and abnormal cells in root tips from maize seeds irradiated while dormant with X rays and thermal neutrons, 1954 irradiation series.

Treatment	No. of root tips sampled	No. of cells	Bridges		Fragments		Abnormal cells[1]	
			No.	Per cell	No.	Per cell	No.	Percent
X rays								
Control	16	392	7	.02	7	.02	9	2.3
5,800 r	11	507	275	.54	425	.84	206	40.6
8,120 r	13	566	404	.71	589	1.04	296	52.3
10,440 r	13	532	489	.92	816	1.53	357	67.1
11,948 r	17	465	579	1.25	1072	2.31	367	78.9
Thermal neutrons per square centimeter								
Control	8	495	5	.01	7	.01	10	2.0
1.6×10^{13}	14	509	627	1.23	1073	2.11	397	78.0
2.3×10^{13}	12	508	771	1.52	1443	2.84	433	85.2
2.5×10^{13}	11	509	790	1.55	1519	2.98	444	87.2

[1] Cells which had bridges and/or fragments.

Table 16.—Frequencies and types of chromosomal interchanges at meiosis in plants from irradiated seeds, 1954 irradiation series.

Treatment	Interchange frequency[1]			Type of interchange Frequency per 100 plants			
	No. of plants	No.	%	Ring-of-4	Ring-of-6	Two rings-of-4	Others[2]
X rays							
Control	90	0	0.0	0	0	0	0
5,800 r	185	15	8.1	8.1	0	0	0
8,120 r	190	11	5.8	5.8	0	0	0
10,440 r	177	20	11.3	9.0	0	1.1	0
11,948 r	167	41	24.6	24.6	0	0	0
Thermal neutrons per square centimeter							
Control	84	0	0.0	0	0	0	0
1.6×10^{13}	190	95	50.0	30.0	5.3	4.7	3.3(A) 0.5(B)
2.3×10^{13}	180	67.5	37.5	25.6	1.7	3.3	0.6(B) 0.6(D)
2.5×10^{13}	170	97.5	57.3	37.6	2.9	5.7	0.6(A) 0.6(C) 0.6(D) 0.6(E)

[1] Using a ring-of-4 as a basis. A ring-of-6 is counted as 1.5 rings-of-4, a ring-of-8 as two rings-of-4, etc.

[2] These include:

A. Two pseudo-isochromosomes
B. One ring-of-8
C. Two rings-of-6
D. Three rings-of-4
E. One ring-of-4 + one ring-of-8

the anaphase stage for the presence of chromatin bridges and fragments. The summary of these observations is given in Table 15. The period between irradiation and germination of the seeds was 381 days for the X-ray series and 410 to 420 days for the different doses in the thermal neutron series. The data in Table 15 indicate that the highest X-ray dose and the lowest thermal neutron dose have produced comparable frequencies of bridges, fragments and abnormal cells. Most of the bridges and fragments occurred in pairs.

The frequency of chromosomal interchanges in meiotic cells was determined for plants grown from irradiated seeds in the summer of 1954, and the results are given in Table 16. An attempt was made to get a sample from the first 100 plants of each control and from the first 200 plants of each treatment. This was achieved with few exceptions, but some of the samples were not usable because of improper stages of meiosis or poor quality, which accounts for the discrepancies between the objectives and the number of plants shown in Table 16.

In comparing Tables 15 and 16 it is apparent that the increase in frequency of chromosomal aberrancies with increases in dosage is not as consistent for the meiotic interchanges as for the mitotic bridges and fragments. The implications will be considered in the discussion. Another comparison worth noting is that, for mitotic aberrations, the highest X-ray dose and the lowest neutron dose gave comparable effects, whereas for meiotic interchanges the highest X-ray dose had considerably less effect than the lowest neutron dose. It is possible, however, that storage of the X-rayed seeds for a year before obtaining the mitotic aberration frequencies had caused an increase in those aberrations.

Transmitted Mutations

In order to obtain information on the segregation of seedling mutations in the R_3 generation, the R_1 and R_2 generations were handled as described on page 10. For each R_2 progeny 21 seeds, or all available, were planted.

The frequencies of R_2 progenies segregating for seedling and mature plant mutations are given in Table 17 and the frequencies of different types of seedling mutations in Table 18. Only R_2 progenies containing ten or more plants are included. In this generation only the highest dose of X rays and of thermal neutrons had a significant increase over its control in the frequency of seedling mutations. As for specific types of seedling mutations (Table 18), there was a significant increase over the control in necrotic seedlings for the highest X-ray dose and in both virescent and glossy for 2.3 x 10^{13} thermal neutrons per square centimeter. This thermal neutron dose was the only radiation treatment which had a significant increase over the control in the frequency of mutations appearing in the mature plants. The increase was due almost entirely to the segregation of a male-sterile phenotype in five different R_2 progenies. All the radiation doses had at least one progeny segregating for male sterility, whereas neither of the controls had any. Other mutations noted in the more mature phases of plant development included liguleless, tasselless, "lazy" and brown midrib, none of which occurred in the controls, and short internodes, narrow leaves and stripes, which occurred in the controls and some radiation treatments.

Table 17.—Mutation frequencies in R_2 and R_3 progenies, 1954 irradiation series.

Treatment	R_2 progenies			R_3 progenies	
	No.	Seedling mutations per 100 progenies	Mature plant mutations per 100 progenies	No.	Seedling mutations per 100 progenies
X rays					
Control	103	22.3	3.9	200	19.0
5,800 r	159	23.9	3.1	172	26.7
8,120 r	150	17.3	2.0	174	31.6**
10,440 r	211	20.4	6.2	219	37.0**
11,948 r	145	34.7*	7.6	155	41.3**
Thermal neutrons per square centimeter					
Control	119	21.8	5.0	240	18.7
1.6 x 10^{13}	68	27.9	2.9	157	35.7**
2.3 x 10^{13}	42	26.2	14.3*	170	32.9**
2.5 x 10^{13}	84	34.5*	7.1	181	38.1**

*Significantly different from control at the 5% level of significance.
**Significantly different from control at the 1% level of significance.

Table 18.—Percentages of R_2 and R_3 progenies with different types of seedling mutations, 1954 irradiation series.

Treatment	Type of mutation											
	White	Cream	Yellow	Yellow-green	Virescent	Striate	Stripes	Mutable	Necrotic or chlorotic	Glossy	Dwarf	Others
Control for X rays												
for R_2	3.9	2.9	0.0	11.7	1.0	0.0	1.0	0.0	1.9	0.0	0.0	0.0
for R_3	1.5	2.5	1.0	7.0	3.0	0.0	0.0	1.5	1.5	0.0	0.0	1.0[1]
5,800 r												
for R_2	1.3	6.9	1.3	7.5	0.0	0.6	3.8	0.0	1.3	0.6	0.6	0.0
for R_3	0.6	5.8	1.2	5.2	7.0	1.2	0.6	2.3	1.2	0.0	0.6	1.2[1]
8,120 r												
for R_2	0.0	3.3	0.7	6.7	2.0	0.0	1.3	0.0	3.3	0.0	0.0	0.0
for R_3	0.6	6.9	0.6	5.2	10.9	0.0	1.7	1.7	2.3	1.7	0.0	0.0
10,440 r												
for R_2	2.4	0.0	0.5	5.2	5.7	0.0	2.4	0.0	4.3	0.9	0.5	0.0
for R_3	0.5	6.8	0.9	8.2	14.6	0.0	0.0	1.4	0.5	1.4	0.0	1.4[1] 0.9[2]
11,948 r												
for R_2	6.2	2.1	0.0	13.2	3.5	0.0	0.7	0.0	8.3	0.7	0.0	0.0
for R_3	0.6	7.1	0.0	8.4	15.5	0.0	1.9	0.0	3.9	0.6	0.0	1.9[1] 0.6[2]
Control for thermal neutrons												
for R_2	1.7	3.4	0.0	5.9	0.8	0.0	3.4	0.0	5.0	0.8	0.8	0.0
for R_3	1.2	4.6	0.4	3.3	5.4	0.0	0.4	2.1	0.8	0.0	0.0	0.4[1]
1.6 x 10^{13} $N_{th.}$ per square centimeter												
for R_2	1.5	0.0	1.5	4.4	0.9	0.0	1.5	0.0	7.4	0.0	0.0	2.9[1]
for R_3	0.0	6.4	2.5	12.7	3.8	0.0	0.6	1.9	3.8	1.3	0.6	1.3[1]
2.3 x 10^{13} $N_{th.}$ per square centimeter												
for R_2	2.4	2.4	0.0	7.1	4.8	0.0	0.0	0.0	4.8	4.8	0.0	0.0
for R_3	0.6	7.6	1.2	6.5	6.5	0.0	1.2	1.2	1.8	4.1	0.0	1.8[1] 0.6[2]
2.5 x 10^{13} $N_{th.}$ per square centimeter												
for R_2	1.2	6.0	2.4	10.7	2.4	0.0	1.2	0.0	9.5	0.0	1.2	0.0
for R_3	1.1	6.1	1.7	12.2	5.5	1.1	1.1	1.7	5.5	0.0	1.7	0.0

[1] Fused leaves
[2] Olive-green seedlings

An attempt was made to get from 175 to 200 R_3 progenies per treatment. In order to satisfy this aim for the two controls and the thermal neutron series, self-pollinated ears from two different plants in each R_2 progeny were used. In some cases both ears segregated for the same seedling mutation based on phenotypic appearance. Where this occurred in the controls, it was most likely due to recessive genes in the stock. In the thermal neutron treatments such an occurrence could be due to either an inherent mutation or an induced mutation occurring in two sister R_2 plants. However, even if induced, the chances that the two mutations have independent origins is remote. Therefore, to avoid any possible duplication of a single mutational event, similar mutations occurring in both R_3 progenies from R_2 sister plants were counted as one inherent or induced mutation.

The frequencies of R_3 progenies segregating for seedling mutations are shown in Table 17, and the frequencies of different types of mutations in Table 18. Only R_3 progenies having ten or more plants were included, which accounts for the fact that some treatments have less than the desired number of progenies.

Except for the 5,800 r X-ray dose, all radiation treatments had significantly greater frequencies of progenies segregating for seedling mutations than the controls. In comparing the various radiation doses with the controls for segregations of specific mutations (Table 18), there was a significant increase in cream and virescent at all X-ray doses, in yellow-green and necrotic at the lowest and highest thermal neutron doses, and in glossy at the dose of 2.3 x 10^{13} thermal neutrons per square centimeter.

In comparisons between the R_2 and R_3 generations for total mutation frequency, the only significant increases in the R_3 were for the X-ray doses of 8,120 r and 10,440 r, although each radiation dose had a higher frequency of mutations in the R_3 than in the R_2. There were no significant differences between the R_2 and R_3 generations for either of the controls in overall mutation frequency, but there was a significant increase in virescent in the R_3 for the thermal neutron control, along with a significant decrease in stripes and necrotic plants. There were significant increases in virescent in the R_3 at all X-ray doses and in cream at the two highest X-ray doses. The only significant increases in specific mutations in the R_3 for the thermal neutron doses were in cream and yellow-green at the lowest dose.

In the R_3 compared with the R_2 there were significant decreases in stripes for 5,000 r and 10,440 r units and in necrotic for 10,440 r units of X rays. The decrease in stripes may be due to the fact that the R_2 plants were observed throughout their development rather than for only two or three weeks as in the case of the R_3 plants, so that some of the stripes recorded in the R_2 were of the type which develop after the seedling stage.

DISCUSSION

The consecutive series of experiments reported in this bulletin indicate some of the problems which may be encountered in growing field populations from irradiated seeds for quantitative studies of irradiation effects. When treated maize seeds, except for those exposed to low doses, were planted directly in the field, they were more sensitive than untreated seeds to adverse environmental effects at the time of emergence. These conditions occurred with the May 15, 1952 planting and the 1953 spring planting. In the 1954 series the seedlings emerged and became established under greenhouse conditions, and the use of plant bands resulted in a minimum of disturbance when they were transplanted to the field. Therefore, it is likely that the mature stands for this series (Table 11) indicate irradiation effects more accurately than those of any other series.

The dose ranges used in the 1954 series were based on the results of the previous tests and were chosen to give adequate number of mature plants for determinations of meiotic chromosomal aberrations and transmitted mutations. The earlier series had shown that, for seeds planted directly in the field, the upper dose limits would be around 12,000 r units of X rays and 2.5 x 10^{13} thermal neutrons per square centimeter (see Tables 1 and 7). The 1954 series (Table 11) indicates that, by using larger seed lots with increasing dosage and by initiating seedling growth in a greenhouse, it would be possible to increase the dose range to some extent for thermal neutrons and to a greater extent for X rays, and still have comparable numbers of mature plants at each dose.

The data presented in Tables 3 and 4 indicate that plants in which interchanges were observed at meiosis matured later than those without interchanges. These aberrations would not be expected to affect the rate of division of the somatic cells, since they represent the symmetrical type of interchange, and the chromosomes should behave regularly at mitotic divisions. However, deficiencies induced as a result of the breaks which were involved in the interchanges could prolong the rate of cell division and cause a delay in maturity. Meiotic cells were not observed for the presence of deficiencies, but the types of aberrations scored in the root-tip cells in the 1954 series (see Table 15) would lead to deficiencies. Many of these cells would be too severely affected to proceed through many division cycles in competition with cells affected to a lesser extent or not at all. However, the descendants of cells with fewer or smaller deficiencies might conceivably persist through the life of a plant, although the rate of cell division could be retarded.

In comparing the frequencies of root-tip chromosomal aberrations (Table 15) with the meiotic interchange frequencies (Table 16), it was noted that the root-tip aberrations showed a more consistent relationship with dosage than the meiotic aberrations. The cells observed in the root tips were believed to be in the first cycle of division after the germination of the seeds. Hence, the complete picture of irradiation effects was observable. Between this stage and the meiotic stages many cell generations would have elapsed and, as mentioned above, the more severely affected cells would have either been killed or outstripped in division by the more normal cells. Any interchanges occurring in these damaged cells would not be recorded at the time of meiosis. Furthermore, the tassels of plants from irradiated seeds were likely composed of sectors of cells with and without interchanges. Since only a few cells of each tassel were observed, the sectors with interchanges could be missed as a result of chance. For these reasons discrepancies between the two sets of data would be expected.

One of the effects of irradiating dormant maize seeds was a branching of the main stalk in 57 R_1 plants of the 1952 series and in three R_1 plants of the 1954 series. A similar effect has been observed in maize by Saric (1958a), in the apple by Bishop and Aalders (1955) and in the tomato by Yagyu and Morris (1957).

Saric (1958a) described three types of branching in maize inbred lines or hybrids as a result of X-ray irradiation. One type, which seems similar to the branching described in the current study, involved fused growth of two stalks to the fourth internode, at which point the stalks separated. They were designated as twin stalks because they had the same number of internodes, leaves and ears at parallel heights. Other types of branching observed included the growth of a lateral branch from a single node or the growth of lateral branches from a number of nodes. The author gave no explanation for the branching other than the effect of irradiation on the embryonic meristem, but he did point out the practical significance of increasing the number of ears on a plant through branching.

In reporting on shoot bifurcation in the apple, Bishop and Aalders (1955) suggested that induced chromosomal aberrations could cause the death of a cell or group of cells in a critical location, resulting in a change in the polarity of cell division for a short time. Shoot bifurcation would thus be due to the delayed expression of a chromosome effect. The authors found that, for doses of X rays and thermal neutrons which gave the same degree of inhibition of shoot growth, thermal neutrons produced approximately twice as many shoot bifurcations per inch of growth as X rays. They attributed this difference to the effectiveness of thermal neutrons in breaking chromosomes. In the observations on maize reported in this bulletin, most instances of branched stalks were induced by thermal neutron radiation, which would support the observations and suppositions of these authors.

The frequencies of seedling mutations in the R_2 and R_3 generations should determine the segregating generation for induced mutations (Tables 8, 9 and 17). Unfortunately, one-fourth to one-fifth of the control plants of the single cross hybrid, L289 x I205, were heterozygous for recessive genes which segregated after self-pollination. Statistical tests indicated that these inherent recessive genes would account for nearly all the seedling phenotypic changes observed in the R_2 generation, and that most of the mutations resulting from irradiation segregated in the R_3 generation. In the R_2 generation there was a significant increase over the control in seedling mutation frequency for the 10,000 r X-ray dose of the 1953 series and for the highest X-ray and thermal neutron doses of the 1954 series. In these cases some induced mutations must have been expressed in the R_2 generation. They could be due either to a dominant effect or to the occurrence of the same recessive mutation in both the male and female gametes of a plant. However, if due to dominant mutations, some effects should have appeared in the R_1 plants. Since none were observed it is likely that recessive mutations were involved.

Irradiation studies have provided indirect evidence concerning the derivation of the tassel and ear shoot from embryonic cells. Stadler (1931) indicated that, after seed irradiation, sectors of tissue with induced mutations would occur in both the ear and the tassel, suggesting that each of these organs was derived from more than one cell in the mature embryo. However, he stated that a sector including part of the ear might not extend into the tassel and, if it did, the styles of the affected portion of the ear would be pollinated with a mixture of pollen from affected and unaffected parts of the tassel. Anderson *et al.* (1949) determined the frequency and size of tassel sectors of abnormal pollen on plants from irradiated seeds. They estimated that seven or eight cells present in the growing tip of the irradiated, dormant seed were represented in the reproductive cells of the tassel on the main stalk.

None of the observations in this study would aid in determining the number of embryonic cells involved in the tassel and ear shoot. However, the information on the occurrence of seedling mutations in the R_2 and R_3 generations may indicate whether or not the male and female organs have a common origin. Since, according to statistical tests, most of the induced mutations did not appear in the R_2 generation, the male and female gametes which combined in the R_1 plants usually did not have mutations in common. This would suggest that the tassel and ear shoot originated from different cells in the embryo of the mature seed. An alternative explanation is that the male and female organs are derived from common cells in the embryo, but that the sectors having mutations are small in both organs, so that the chances for union of gametes with common mutations are remote.

It is of interest to compare some of the observations made on maize in the current study with those reported for barley (Caldecott *et al.*, 1952, 1954) and for tomato (Yagyu and Morris, 1957). While such comparisons should be made with some reservations for crops irradiated and grown at different times, these three studies do have certain

Table 19.—Comparison of frequencies of induced chromosomal aberrations and seedling mutations in barley, tomato and maize.

Irradiation effect	10,000 r X rays			1.5 x 10^{13} thermal neutrons per cm.[2]		
	Barley	Tomato	Maize	Barley	Tomato	Maize
Percent mitotic cells with bridges and/or fragments						
Short storage period[1]	34.2	64.0	...	81.0	41.7	...
Long storage period[2]	...	80.1	64.8	...	...	76.0
Frequency of meiotic interchanges per 100 cells	13.6	...	11.3	87.5	...	50.0
Frequency of seedling mutations per 100 progenies	3.6	11.6	18.0	12.5	16.6	17.0

[1] Not over 40 days between irradiation and germination.
[2] Period between irradiation and germination 322 days for X-rayed tomato, 381 days for X-rayed maize, 410 days for thermal neutron-irradiated maize.

common aspects, since all irradiations were made at the Brookhaven National Laboratory and the data were obtained from plantings made at Lincoln, Nebraska, with an overlapping of personnel making the observations.

An X-ray dose of approximately 10,000 r units and a thermal neutron dose of approximately 1.5 x 10^{13} thermal neutrons per square centimeter were used in all three studies. Therefore, the effects of these doses may be compared among the three crops with respect to frequencies of mitotic chromosomal aberrations and seedling mutations, and between barley and maize with respect to meiotic interchange frequencies (Table 19). Only the 1954 maize irradiation series is used in this table. In making comparisons the frequencies of aberrations or mutations in the control have been subtracted from those in the treatments for each crop.

For mitotic chromosomal aberrations observed after a comparatively short storage period between irradiation and germination, there were twice as many aberrant cells in tomato as in barley for the X-ray dose, whereas the inverse relationship held for the thermal neutron dose. The mitotic X-ray data for maize should not be compared directly with the results in tomato and barley because of a possible storage effect on aberration frequency. This is indicated by the two sets of X-ray data in tomato. Seeds germinated ten days after irradiation gave 64 percent abnormal mitotic cells, whereas those stored for 322 days before germination gave 80.1 percent abnormal cells, an increase of 16 percent. Maize seeds held for 381 days before germination gave 64.8 percent abnormal mitotic cells. Assuming a storage effect in maize comparable to that in tomato, it would seem that, using mitotic aberrations as a measure, the reaction of the three crops to the X-ray dose in order of increasing resistance was: tomato, maize, barley. The results for the thermal neutron dose do not permit any conclusions concerning the effects of storage in the case of maize. Assuming that there is little, if any, storage effect (Curtis *et al.*, 1957), the reaction to the thermal neutron dose in order of increasing resistance would be: barley, maize, tomato.

Unfortunately, meiotic interchange frequencies after irradiation of tomato seeds were not obtained. For barley and maize the interchange frequencies for the X-ray dose differed by less than 3 percent, but for the thermal neutron dose barley had a 37 percent increase over maize.

The seedling mutation frequency for the X-ray dose was highest for maize and lowest for barley, with tomato having an intermediate frequency. The frequencies for the thermal neutron dose showed less difference among the three crops; however, barley showed the least effect.

The observations summarized in Table 19 indicate that the three crops may be ranked differently in their reaction to irradiation, depending on the type of radiation used and the specific effect observed. These are important considerations in comparing the relative resistance or sensitivity of different crops.

A comparison of the relative frequencies of the different types of induced meiotic interchanges in barley and in maize is presented in Table 20. The data for barley are taken from Table 5 in Caldecott *et al.* (1954), and those for maize include the 1952 and 1954 irradiation series (see Tables 5 and 16 in this bulletin). All doses of both X rays and thermal neutrons have been combined for each crop.

Caldecott *et al.* (1954) found that in barley the two sources of radiation produced statistically different relative frequencies of the various types of interchanges. Similar differences were indicated for the maize data shown in Table 20 when tested by a chi square in a 2 x 7 contingency table ($P<.01$). In barley the major contribution to the total chi square came from rings-of-six and a ring-of-four plus a ring-of-six, and in maize from rings-of-six and two rings-of-four. In both crops there was a relatively higher frequency of the more complex types of interchanges in the thermal neutron series than in the X-ray series.

The distributions of the different types of interchanges in barley

Table 20.—Comparisons of the relative frequencies (%) of different types of meiotic interchanges induced in barley and maize by X rays and thermal neutrons (compiled from Caldecott *et al.*, 1954, and the 1952 and 1954 irradiation series of the current study).

Type of radiation and crop	Total no. of inter-changes	Ring-of-4	Ring-of-6	Ring-of-8	Two rings-of-4	Two pseudo-isochro-mosomes	Ring-of-4 + ring-of-6	Others
X rays								
Barley	297	84.8	3.7	1.0	6.1	4.0	0.0	0.3
Maize	216	83.8	3.2	1.4	6.9	1.9	0.5	2.3
Thermal neutrons								
Barley	657	74.0	10.2	1.8	7.9	3.2	1.5	1.4
Maize	588	71.3	8.3	1.4	14.5	1.2	0.7	2.4

and maize may also be compared for each type of radiation. The chi square test indicated a similar distribution in the two crops for the X-ray-induced interchanges ($.10<P<.25$), but a different distribution for the thermal neutron series ($P<.005$). In the neutron series the two noticeable differences involve two rings-of-four, which are almost twice as frequent in maize as in barley and the pseudo-isochromosomes, resulting from breaks and reunions in homologous chromosomes, which are more frequent in barley than in maize.

The relative frequencies of different kinds of induced seedling mutations in barley (Caldecott *et al.,* 1954, p. 249), tomato (Yagyu and Morris, 1957, p. 233), and maize (current study, Tables 10 and 18) are shown for the two types of radiations in Figure 13. All doses used were combined in each case. The four types of chlorophyll mutations which were designated by the same name for the three crops are shown separately. All other mutations are grouped under the class marked "others." In presenting the data for maize, the frequencies of the various mutant types in the controls have been subtracted in determining the induced frequencies.

The distribution of the five classes of mutations is remarkably similar for the two sources of radiation in the case of barley and of tomato. In maize the X-ray series had no yellow or yellow-green mutants, but the thermal neutron series had 4.6 and 21.5 percent, respectively, of these classes. There were no virescent mutants in the thermal neutron series, but 38.1 percent in the X-ray series.

There were pronounced differences among crops with respect to the frequencies of specific mutant types. The white mutants made up over half the seedling mutation spectrum in barley, but only a small part of the mutant types in tomato and none of those in maize. Virescent constituted a much larger proportion of the total mutants for the X-ray series in maize than in barley or tomato. The four classes of chlorophyll mutations shown separately constituted almost 90 percent of the seedling mutant types in barley, but less than 50 percent of those in tomato and maize.

The seedling studies on all three crops were made in the greenhouse, but at different times. Therefore, it is likely that environmental factors, as well as the subjective method of classification, contributed to some of the differences observed among crops. However, the striking difference among crops in the frequency of white mutants, which should be least affected by the factors mentioned above, indicates that the crops responded differently to irradiation in this regard. As pointed out by Caldecott *et al.* (1954), each of the chlorophyll mutant classes probably involves changes in groups of genes rather than in specific genes. It is apparent that the types of mutations which result in the complete absence of chlorophyll occur much more frequently in barley than in tomato or maize.

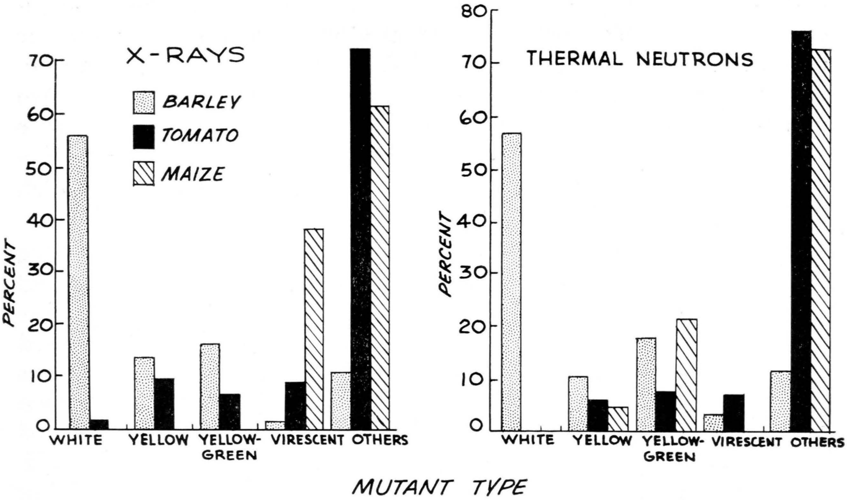


Figure 13. Relative frequencies of different types of induced seedling mutations in barley, tomato and maize.

SUMMARY

Dormant maize seeds of the single cross hybrid L289 x I205 were irradiated with various doses of X rays and thermal neutrons in 1952, 1953 and 1954. The X-ray doses during the three years ranged from 4,000 r to 32,000 r units, and the thermal neutron doses from 5.8×10^{12} to 5.1×10^{13} thermal neutrons per square centimeter.

In the 1952 irradiation series, in which the seed was planted directly in the field, there was a marked drop in stands between 8,000 r and 16,000 r units for X rays and between 2.5×10^{13} and 4.2×10^{13} thermal neutrons per square centimeter. This information was used in selecting the dose ranges for future experiments.

Phenotypic changes in the R_1 plants of the 1952 series included chlorophyll sectors, some of which were mottled, slashed leaves and branched stalks.

The low spontaneous frequency of interchanges was markedly increased by both X-ray and thermal neutron irradiations. For some of the treatments a statistically significant difference in interchange frequency was obtained for the two planting dates. Since the plants with interchanges tended to mature later than those without interchanges, possibly due to concurrent deficiencies, a non-random method of sampling resulted in selecting the plants which matured earlier at the first planting date. Because of smaller stands at the second planting date, more of the later-maturing plants were sampled and a higher interchange frequency was obtained.

The types of interchanges observed in the 1952 series included two

cases with a ring-of-ten plus a ring-of-four, involving a minimum of seven breaks. There were no statistically significant differences between the combined X-ray and the combined thermal neutron doses with respect to the frequencies of the different types of interchanges except in the case of two-rings-of-four, which occurred more frequently in the thermal neutron series.

The frequencies of induced abnormal pollen in the 1952 series ranged from 8 percent for the lowest dose of X rays to 75 percent for the highest dose of thermal neutrons. A more uniform effect among plants was noted for the highest thermal neutron dose than for the highest X-ray dose.

Seed of the 1953 spring irradiation series was planted directly in the field, but adverse weather conditions after emergence resulted in almost complete killing for all except the lowest doses.

Seed of the 1953 fall irradiation series was planted in the field in Florida. Owing to much higher flux values of thermal neutrons than intended at the time of irradiation, the stands were drastically reduced for all except the lowest dose. However, for this treatment and the X-ray series, the frequencies of seedling mutations were recorded in the R_2 and R_3 generations. The R_2 data disclosed that the single cross hybrid L289 x I205 was heterozygous for a number of recessive genes. After making allowance for these inherent segregations, the 10,000 r X-ray dose was the only treatment with a statistically significant increase over the control in frequency of R_2 progenies with seedling mutations. None of the treatments differed significantly from the control with respect to mature plant mutations in the R_2 generaion.

In the R_3 generation there were statistically significant increases over the control in seedling mutation frequency for the 10,000 r X-ray dose and for the dose of 2.2×10^{13} thermal neutrons per square centimeter. There also were significant increases for specific mutants in some treatments when compared with the control. There was no significant difference in seedling mutation frequency between the controls of the R_2 and R_3 generations, but there were significant increases in the R_3 for the three irradiation doses, both with respect to over-all mutations and some specific types.

Seed of the 1954 irradiation series was planted in bands of soil in flats in the greenhouse and transplanted to the field after seedling establishment. Increasing numbers of seeds were used with the higher doses, in order to assure a minimum mature stand of 500 plants for each treatment, which was achieved. Additional lots of irradiated seeds and controls were germinated for observations on root-tip chromosomal aberrations and seedling development.

Measurements of plant height on 12- to 15-day-old seedlings were taken in June and November, 1954, and in June, 1955. Statistical analyses indicated significant differences between the control and each treatment in all three tests and between some of the treatments. A comparison of the two tests made in 1954 indicated that for the X-ray series plant height was depressed to a greater extent in the second test than in the first, whereas in the thermal neutron series there was less difference between the two tests. This may be an indication of the differences in the effects of storing X-ray and thermal neutron irradiated seeds.

The frequencies of chromatin bridges and fragments were determined in root-tip cells from seeds germinated 381 days after X-irradiation and 410 to 420 days after thermal neutron irradiation. Most of the bridges and fragments occurred in pairs. The frequencies of abnormal cells (including bridges and/or fragments) were around 2 percent in the controls and ranged from 40.6 to 78.9 percent for the X-ray series and from 78.0 to 87.2 percent for the thermal neutron series. It is possible that there was a storage effect in the case of the X-ray series.

The frequency of chromosomal interchanges in meiotic cells did not show as consistent a relationship with dosage as the root-tip aberrations. This was believed due to the death or lag in division of the more severely damaged cells during the many cell generations between the first mitotic cycle in the root and meiosis in the anthers. There were only two cases of the more complex type of interchange in the X-ray series compared with a number of cases in the thermal neutron series.

In the R_2 generation the highest dose of X rays (11,948 r) and of thermal neutrons (2.5 x 10^{13} thermal neutrons per square centimeter) had a significant increase over the control in frequency of seedling mutations. Only the dose of 2.3 x 10^{13} thermal neutrons per square centimeter had a significant increase over the control in mature plant mutations and this was due mainly to the segregation of a male-sterile phenotype. Other mature plant mutations which did not occur in the controls included liguleless, tasselless, brown midrib and a phenotype similar to the "lazy" character.

In the R_3 generation all treatments except the lowest X-ray dose (5,800 r) had significant increases over the control in over-all frequencies of seedling mutations. Some treatments showed significant increases over the control with respect to specific mutations. There was no significant difference in over-all mutations between the controls of the R_2 and R_3 generations, and, of the treatments, only two X-ray doses had significant increases in the R_3 over the R_2. For some treatments there were significant increases in specific mutations in the R_3.

Since most of the induced seedling mutations did not segregate until the R_3, it is suggested that the tassel and ear shoot may originate from different cells in the embryo of the mature seed. An alternative explanation is that they are derived from common cells, but have small sectors containing mutations, so that in the R_1 plants the chances for union of gametes having the same mutation are remote.

A comparison of the data obtained for maize wth similar data obtained for barley and tomato in separate studies indicated that the three crops may be ranked differently in their reaction to irradiation depending on the type of radiation used and the specific effect observed. For barley and maize there was a similar distribution of the different types of interchanges in the X-ray series, but a different distribution for the thermal neutron series, particularly with respect to two rings-of-four and pseudo-isochromosomes. There were pronounced differences among the three crops with respect to the frequencies of specific seedling chlorophyll mutants.

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